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ORIGINAL ARTICLE

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Estimation of postmortem metabolic changes in porcine brain tissue using ^{1}H-MR spectroscopy—preliminary results

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Abstract To investigate the potential for estimating the time since death by monitoring the evolution of different metabolites in brain tissue by ^{1}H-MRS, an animal model using pig heads was established. The maximum examination interval was 3 weeks. Within this time interval spectra revealed different compositions of metabolites, including metabolites observed in the normal brain and as products of bacterial decomposition processes (N-acetyl-aspartate 0–130 h, creatine 0–170 h, bound trimethylammonium, e. g. choline compounds, during the whole time course with fluctuating intensities, lactate 0–200 h, alanine and acetate during the whole time course, succinate and free trimethylammonium after approx. 100 h postmortem). The proposed approach may offer a new method to estimate later postmortem intervals although these observations have to be confirmed by further studies.

Keywords ^{1}H-magnetic resonance spectroscopy · Animal model · Brain · Postmortem interval

Introduction

For the estimation of the early postmortem interval (PMI) 1 up to 1.5 days some methods are well established [5]. For the later postmortem interval, the number of methods is much smaller and their accuracy is clearly lower. This is even true for new entomological methods [1, 2, 3, 7, 10]. Therefore, reliable and reproducible methods to determine the time since death for the later postmortem interval are still required. First results by using proton magnetic resonance spectroscopy (^{1}H-MRS) have been presented with brain tissue data from a sheep model [6]. The essential observation was that after 3 days new metabolites of autolysis and putrefaction appeared in the brain.

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Material and Methods

Method

^{1}H-MRS is an ideal method for storage experiments because it is possible to examine the same intact brain repeatedly. Brain tissue was selected due to its comparably stable, isolated and protected location in the intact skull. We decided to use a pig model which has already proven to be useful for brain examinations [4].

Experimental setting

Five isolated whole heads of young pigs from an abattoir with known time of death were prepared by closing the spinal canal with plasticine, fixing in a plastic holder and storing within a plastic bag with a tube system for instilling fresh air. Storage temperature was kept constant at 21±1°C. ^{1}H-MRS was performed with a clinical 1.5 T whole body scanner using a quadrature head coil. Spectra were acquired by using a PRESS-sequence (point resolved spectroscopy) with water suppression (TR/TE=1500 ms/135 ms, voxel size: 3–6 ml). The voxel position was controlled by T_1-weighted imaging. The time intervals between measurements varied between 8 to 48 h depending on the availability of the MR-scanner.

Results

Gas bubbles occurring in the brain tissue after days 5–7 complicated the selection of voxels with homogeneous brain tissue and impaired spectroscopic measurements. Therefore in 3 of the 5 cases the experiments had to be terminated after 4.4–9.3 days. During short PMIs spectra

showed signals of healthy living brain, like the singlets of N-acetyl-aspartate (NAA) at 2.01 ppm, creatine (Cr) at 3.02 ppm, and a single peak of bound trimethylammonium compounds (TMA), which is mainly composed of choline, phosphocholine and glycerophosphocholine at 3.19 ppm [9]. In addition, doublets of lactate (Lac) at 1.31 ppm and alanine (Ala) at 1.47 ppm appeared in the first spectra. In the first spectra acetate (Ace) was observed only as a small shoulder of the NAA peak. Between 70 and 100 h postmortem the intensity ratio between NAA and Ace was reversed and Ace became the predominant peak. At longer PMIs (more than 100 h, 4 days) additional signals evolved at 2.41 ppm (succinate) and 2.88 ppm (free trimethylammonium, fTMA) [6] (see Fig 1 and Table 1) whereas some metabolites disappeared (NAA 130 h, Cr 170 h, Lac 200 h postmortem).

Table 1 Occurrence of the different metabolites which were observed during the different postmortem times based on all five experiments

Metabolite	PMI (h)	Metabolite	PMI (h)
NAA	0–130	fTMA	After 100
Creatine	0–170	Alanine	At all times
Lactate	0–200	Acetate	At all times
Succinate	After 100	TMA	At all times

PMI Postmortem interval.
NAA N-acetyl-aspartate.
fTMA Free trimethylammonium.
TMA Trimethylammonium.

Discussion

Although the very preliminary results presented here are based on a small database, that does not allow precise information about the variance of metabolic postmortem changes to be extracted, we were nevertheless able to demonstrate that postmortem metabolic changes start shortly after death and that these changes can be monitored over time by ^{1}H-MRS. Metabolites which are absent in healthy brain tissue but observable in brain abscesses [8] were detected (e.g. alanine, succinate, fTMA), indicating the genesis of new products. There were three different types of metabolic time courses:

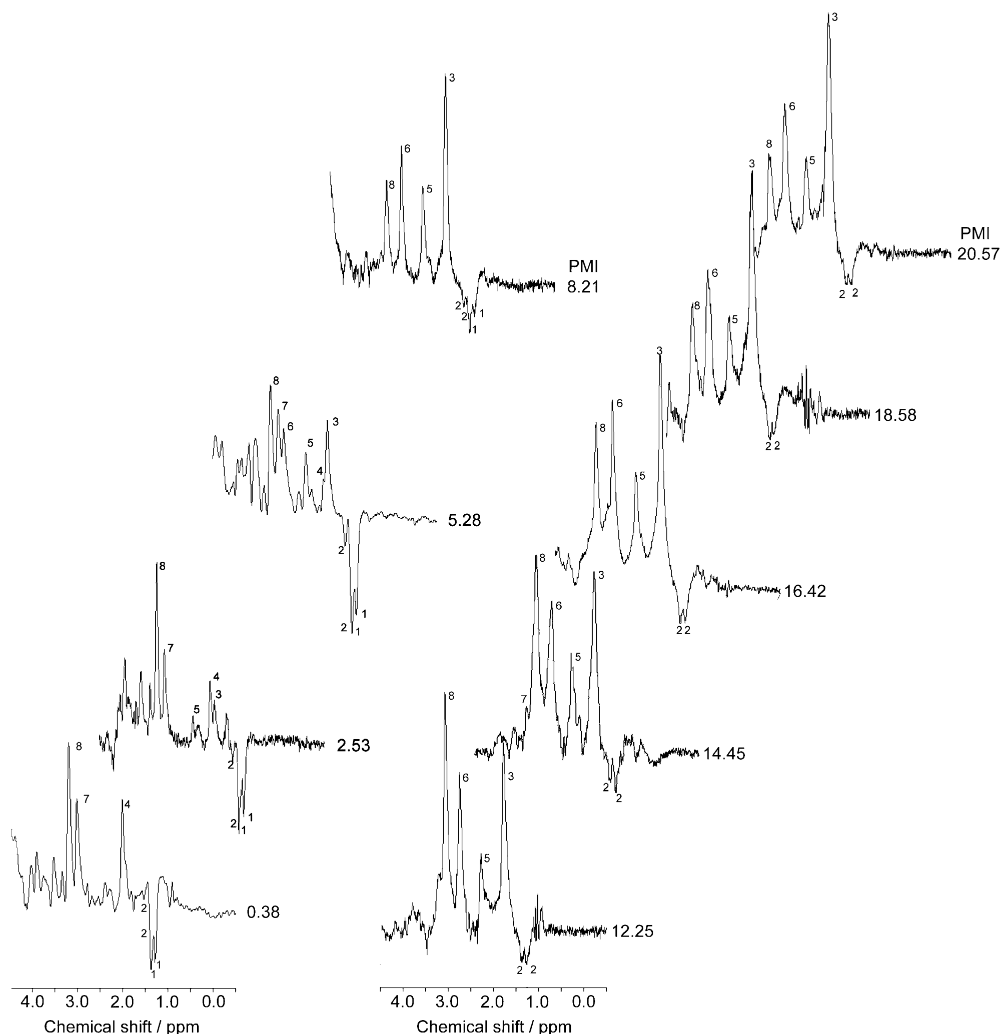

Fig . 1 Time course of spectral changes observed within 3 weeks exemplary for 1 brain (PMI in days). *Peak 1* Lactate (Lac)/1.28/1.37 ppm, *2* alanine (Ala)/1.37/1.53 ppm, *3* acetate (Ace)/1.91 ppm, *4* N-acetyl-aspartate (NAA)/2.02 ppm, *5* succinate (Succ)/2.41 ppm, *6* free trimethylammonium (fTMA)/2.88 ppm, *7* creatine (Cr)/3.02 ppm, *8* trimethylammonium (TMA)/3.20 ppm

- Continuously decreasing and finally disappearing intensity (e. g. NAA)
- Continuously increasing intensity and detection throughout the complete time course (e. g. Ace)
- Rather variable over the time period (e.g. TMA).

Monitoring the appearance of new resonances or the disappearance of some signals may therefore serve as an indicator for the stage of decomposition and therefore the PMI. Comparing the intensities of different metabolites would potentially improve the precision of the estimated postmortem interval provided that the variability is low and/or the temporal evolution is known.

The disappearance of metabolites that usually contribute to the in vivo spectra and the occurrence of new metabolites typical for bacterial decomposition, was also observed by Ith et al. [6] who observed additional compounds due to the short echo time (20 ms vs. 135 ms) or due to their different animal model.

Despite careful control of the storage conditions, such as temperature and ventilation, differences in the appearance and size of the gas bubbles were seen indicating that the processes of autolysis and putrefaction are subject to some variations. These variations may be assigned to humidity, bacterial growth or other environmental factors or individual differences.

Conclusions

There exists a special time course of autolysis and putrefaction in a porcine brain model which can be monitored by ^{1}H-MRS. Except for limitations caused by evolving gas bubbles in the brain tissue, reproducible results could be obtained. Whether and how these results are transferable to human investigations has still to be explored.

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